produced by BC11D and BC233D reacted with BE and non-infected equine erythrocytes by Western blot. These monoclonal antibodies recognizing the 48kDa antigen were found to recognize rhoptry by observation with confocal laser microscope (Fig. 1). Subclass and type of L chain of the monoclonal antibodies produced by these hybridoma clones were determined to be IgG2a and IgG1, respectively, with Amersham isotyping kit (manufactured by Amersham).

Example 3: Screening of cDNA Library of BC Merozoite and Sequencing of cDNA Clone

For a primary antibody, culture supernatant of the monoclonal antibody produced by BC11D recognizing the 48kDa protein prepared in Example 2 was diluted 5-folds with PBS supplemented with 1% bovine serum albumin. As a secondary antibody capable of binding to the primary antibody was used alkali phosphatase-conjugated goat anti-mouse IgG antibody (manufactured by Jackson Immunoresearch Laboratories, Inc.) diluted 20,000-folds with PBS supplemented with 1% bovine serum albumin. The cDNA library obtained in Example 1 was screened immunologically with the primary and secondary antibodies. Positive plaque was recovered and cloned. The obtained cDNA clone BC48 was inserted into pBluescript SK (+) plasmid vector (manufactured by Stratagene Inc.) by *in vivo* excision. Thereafter, the cDNA was cleaved out of the vector with

restriction enzymes and subcloned. The inserted DNA was determined for its nucleotide sequence by the dye primer method using M13 reverse and universal primers (manufactured by Stratagene Inc.) with ABI PRSMTM 377 sequencer (manufactured by Perkin Elmer). The obtained sequence data were analyzed with Gene Works (manufactured by IntelliGenetics, Inc.). As a result, it was found that the gene encoding the 48kDa antigen of BC merozoite had the nucleotide sequence shown in SEQ ID NO: 1 of 1,828 base pairs in full length. The gene was found to contain 1,374 base pairs in full length for a structural gene that encodes the amino acid sequence of the 48kDa protein of BC merozoite as shown in SEQ ID NO: 1.

A plasmid vector pGEX/BC48, i.e. pGEX4T-3 wherein the cDNA clone BC48 was incorporated, after transfection into *E. coli*, has been deposited as Escherichia coli/GST-BC48 at the Fermentation Research Institute Agency of Industrial Science and Technology, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan with accession number: FERM BP-6761 on June 16, 1999.

Example 4: Preparation of Recombinant BC Merozoite

The cDNA inserted in pBluescript SK (+) plasmid vector was then cleaved with EcoRI and XhoI and inserted into pGEX4T-3 plasmid vector (manufactured by Pharmacia Biochemicals Inc.) at the EcoRI and XhoI sites (Fig. 2).

The obtained plasmid vector was transfected into *E. coli* (BL21 strain) and expression was induced with isopropyl-β-D-thio-galactopyranoside (IPTG).

After expression, the suspension (500 ml) of *E. coli* was centrifuged at 6,000 rpm at 4°C for 10 minutes and supernatant was discarded. The sediment was suspended in a sonication buffer (50 mM Tris-HCl (pH 8.0)/50 mM NaCl/1 ml EDTA; 10 ml) and sonicated to rupture cells. To the suspension of ruptured cells was added 10% Triton X-100 at a final concentration of 1%. The mixture was centrifuged at 12,000 rpm at 4°C for 30 minutes and supernatant was recovered. To the supernatant was added 0.2 ml of 50% slurry of Glutathione sepharose 4B beads (manufactured by Pharmacia Biochemicals Inc.) and mixed at 4°C for 30 minutes. The mixture was centrifuged at 3,000 rpm at 4°C for 10 minutes and supernatant was discarded. The sediment was mixed with PBS supplemented with 1 ml of 0.5% Triton X-100 (PBST). The mixture was centrifuged at 5,000 rpm at 4°C for 10 seconds and supernatant was discarded. These procedures were repeated twice and washed. Thereto was added a buffer for suspending thrombin (50 mM Tris-HCl (pH 8.0)/150 mM NaCl/2.5 mM $CaCl_2$; 1 ml) and mixed. The mixture was centrifuged at 5,000 rpm at 4°C for 10 seconds and supernatant was discarded. To the sediment was added 0.5 ml of a dispersion buffer containing thrombin at a